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Biochemical Membrane Lipidomics during *Drosophila* Development

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SUMMARY

Lipids play critical roles in energy homeostasis, membrane structure, and signaling. Using liquid chromatography and mass spectrometry, we provide a comprehensive semiquantification of lipids during the life cycle of *Drosophila melanogaster* (230 glycerophospholipids, 210 sphingolipids, 6 sterols and sterol esters, and 60 glycerolipids) and obtain biological insights through this biochemical resource. First, we find a high and constant triacylglycerol-to-membrane lipid ratio during pupal stage, which is nonobvious in the absence of nutrient uptake and tissue remodeling. Second, sphingolipids undergo specific changes in headgroup (glycosylation) and tail configurations (unsaturation and hydroxylation on sphingoid base and fatty acyls, respectively), which correlate with gene expression of known (GlcT/CG6437; FA2H/ CG30502) and putative (Cyt-b5-r/CG13279) enzymes. Third, we identify a gender bias in phosphoethanolamine-ceramides as a lead for future investigation into sexual maturation. Finally, we partially characterize *ghiberti*, required for male meiotic cytokinesis, as a homolog of mammalian serine palmitoyltransferase.

INTRODUCTION

Biological membranes, in a first approximation, are bilayer arrangements of lipids with embedded and associated proteins. The main classes of lipids found in eukaryotic cells are glycerophospholipids (GPL), sphingolipids (SPL), and sterols (ST). Individual lipids species are determined by specific (stereo)chemical composition (e.g., fatty acyl combination and positioning on a glycerol backbone), and are not uniformly distributed within cellular membranes (van Meer et al., 2008). Instead, tissue enrichment (e.g., sphingolipids in brain, ether-linked lipids in heart), subcellular organization (e.g., cardiolipin in mitochondria), lateral segregation (membrane microdomains), and asymmetric distributions across the bilayers add to the aforementioned complexities. One class of membrane lipid which stands out in structural diversity between biological organisms is the SPL. SPL are formed from the condensation of serine/amino acids and fatty acyl-CoA which leads to generation of sphingoid bases (Pruett et al., 2008), and, ultimately after several biosynthetic steps yields ceramides, the portion of SPL which is anchored in the membrane. In *Saccharomyces cerevisae*, the ceramides occur with phosphoinositol and mannosyl residues (Becker and Lester, 1980). In mammalian cells, the headgroup is decorated instead with phosphocholine (sphingomyelin) or a complex carbohydrate portion (glycosphingolipids) (Merrill, 2011). In *Drosophila*, complex glycosphingolipids and phosphoethanolamine-ceramide (PECer) have been described (Rietveld et al., 1999; Seppo et al., 2000).

SPL play critical roles in many cellular and developmental processes (Stiban et al., 2010; Hannich et al., 2011). SPL are increasingly recognized as key regulators of cell cycle progression/apoptosis as well as growth and cellular metabolism in vertebrates (Bartke and Hannun, 2009). *Drosophila* genetics and developmental biology has provided important insights for SPL in this multicellular organism (Acharya and Acharya, 2005). For example, disruption of SPL biosynthesis has been implicated in the regulation of energy homeostasis and body fat metabolism (Bauer, 2010; Kohyama-Koganeya et al., 2011). The diverse functions of this class of lipids further extend to their regulatory role in phototransduction, brain development, and behavior in the fruit fly (Chen et al., 2007; Acharya et al., 2008; Dasgupta et al., 2009; Kraut, 2011).

Despite the increased appreciation of SPL functions, there are many open questions regarding SPL. In fact, the putative functions of SPL in the regulation of cellular physiology are likely to

go far beyond our current understanding of genetic interactions and developmental traits/phenotypes. Lipid-lipid and lipid-protein interactions are critically controlled by SPL (Simons and Gerl, 2010). Functional interactions between sphingolipids and sterols regulate cellular physiology in yeast (Guan et al., 2009), possibly mediated by specific interactions in a region that is referred to as an "interface," as it falls between the hydrophobic portion and hydrated surface of the membrane (Bithell et al., 2008). Indeed, small changes in the orientation of lipids in this region can affect protein function (Lingwood et al., 2011). Furthermore, it has recently been demonstrated that structural uniqueness is an important factor in mediating the recognition of a single SPL species by a transmembrane protein involved in membrane dynamics and traffic corroborating the findings that specific and relatively small differences in chemical composition of lipids (such as a single double bond), i.e., "fine chemical details," may serve different functions (Contreras et al., 2012).

Analytical biochemistry of *Drosophila* lipids has been described in previous studies (Gamo et al., 1999; Fyrst et al., 2004, 2008; Masood et al., 2010; Hammad et al., 2011; Parisi et al., 2011), but very few comprehensive studies have so far been published (Carvalho et al., 2012). The main aim of this work was to establish such a comprehensive, systematic description of membrane lipids found in *Drosophila* with an emphasis on SPL for the reasons mentioned earlier. We focus on analysis of membrane and storage lipids from extracts of whole animals during the life cycle to establish the dynamics of lipid metabolism during the natural process of organismal development. We further combine genetics with this biochemical resource for identification and partial in vivo characterization of an SPL regulator in *Drosophila melanogaster*.

RESULTS

Liquid Chromatography-Mass Spectrometry Approaches for *Drosophila* Lipids

Synchronized developmental stages of embryos, larvae, pupae, and adult flies were raised and harvested using standard protocols. Samples were then processed for chromatographic separation and mass spectrometry (MS). The extraction method based on two-step liquid-liquid partitioning yields an organic phase containing a complex mixture of lipids present in the starting material that are not covalently attached to the nonsoluble residue.

A combination of single-stage MS and tandem MS (MS/MS), i.e., product ion analysis, neutral loss, and precursor scanning, was used to characterize ion responses (Figure S1 and Table S1 available online). In third instar larvae, approximately 90% of membrane lipid is composed of GPL (Figure S2). This is in contrast to the lipid compositions of yeast (Ejsing et al., 2009) or mammalian cell systems (Dennis et al., 2010), which have been recently determined using MS. The predominant GPL in mammalian cells are phosphatidylethanolamine (PE) and phosphatidylcholine (PC), whereas in *Drosophila*, PE alone represents ~2/3 of GPL (Figure S2; Table 1) (Jones et al., 1992; Dobrosotskaya et al., 2002).

Lipid Changes during Developmental Stages

We next applied our targeted workflow to systematically measure lipidomic variations during fly development. A minimum of two biological replicates were measured for each stage, and the time course was repeated three times with different groups of parental flies which were raised separately. The final average values of the major lipid classes (sum of individual species for each class) are represented as mol% of total membrane lipid (i.e., normalized to the sum of GPL + SPL + ST). The levels of the most abundant GPL (PE and PC) are relatively constant during all developmental stages (Figure 1). Less abundant GPL, e.g., phosphatidic acids (PA), display distinct changes during entry into and exit from the adjacent stages. The levels of membrane-bound SPL with none (Cer), one (HexCer), or two (Hex_2Cer) hexosyl moieties in their headgroups rise drastically at the end of the embryonic stage and at the early pupal stage.

To reveal the dynamics of lipid remodeling during development, the abundance of individual lipid species at each time point is calculated against its moving average (i.e., the average value of all time points; Figure 2A). This differential map illustrates the heterogeneity in lipid species that is not apparent in absolute levels of entire classes (Figure 1), nicely demonstrating the power of MS for high resolution lipid analysis. For instance, the levels of some PC species with short fatty acyl chains (# in Figure 2A) are much higher at the larval stage than the moving average, and this is not the case for PC species with longer and more unsaturated fatty acyls (## in Figures 2A and S3B). This suggests that fatty acyl remodeling may be particularly prominent during development. Additionally, levels of many, if not most, SPL species (Cer, HexCer, Hex_2Cer, PECer) are very low (10-fold or less abundant than the average over all stages) at early embryonic stages (0 hr). We have also determined the number of lipid species that change 1.5-fold or more in their abundances between adjacent time points (Figure 2B). With the exception of SPL (which undergo dramatic changes within the first 18 hr; Figure 1), a roughly corresponding number of GPL and SPL are affected between adjacent stages, with the largest differences of 30% or more (Figure 2C) at boundaries between embryos, larvae, pupae, and adult flies (Figure 2B; red brackets). In contrast, some of the changes within larva, pupa, and adult fly stages are limited to a relatively small number of individual lipid species (e.g., # in Figure 2A; as little as 24 of 454 species between 168 and 192 hr of pupal development; Figure 2B).

Careful examination of this high-density lipidomic information of the developing fly reveals an intricate level of specificity in lipid metabolism. This is particularly overt for: (1) fatty acyl distributions between membrane and nonpolar lipids during development (Figure 3); (2) chemical compositions of SPL in all four developmental stages (Figure 4), and, (3) gender-dependent differences of adult flies (Figure 5).

Fatty Acyl Distribution between Membrane and Storage Lipids during Development

Fatty acyls are building blocks in membrane lipids (GPL and SPL) as well as nonpolar lipids with important storage functions (triacylglycerols [TAG] and sterol esters [SE]). These two forms of lipid pools are expected to be highly dynamic during periods of high feeding (embryos and larvae), low feeding, and morphogenesis (pupae), as well as motion (larvae and adult flies). We find high levels of SE in early embryos suggesting that the ST (and/or SE) content plays important roles in early development.

Table 1. Previous Biochemical Lipid Analyses of *Drosophila* and Comparison with This Study

	Analysis Results for Developmental Stages in Previous Studies												
	Jones et al. (1992)		Stark et al. (1993)	Rietveld et al. (1999)	Gamo et al. (1999)	Fyrst et al. (2004)	Fyrst et al. (2008)	Overgaard et al. (2005)	Masood et al. (2010)	Parisi et al. (2011)	Hammad et al. (2011)	Carvalho et al. (2012)	This Study
Lipids Analyzed	Larva	Prepupa	Adult (Head)	Embryo	Adult	Embryo, Larva, Pupa, Adult	Embryo, Larva, Pupa, Adult	Adult	Adult	Adult	Adult	Embryo, Larva, Pupa, Adult	Embryo, Larva, Pupa, Adult
GPL													
PE	50%–60%	50%–60%	+	–	+	–	–	+	+	–	+	+	+
PC	20%–25%	20%–25%	+	–	+	–	–	+	+	–	+	+	+
PI	7.5%–8.5%	8%–9%	+	–	+	–	–	–	–	–	+	+	+
PS	5%–6%	10%	+	–	+	–	–	–	+	–	+	+	+
PA	8%–10%	6%–8%	+	–	–	–	–	–	–	–	–	+	+
PG	–	–	–	–	–	–	–	–	–	–	+	+	+
CL	–	–	–	–	–	–	–	–	+	–	+	+	+
SPL													
Long chain bases	–	–	–	–	–	+	+	–	–	–	–	–	–
Ceramides	–	–	–	+	–	–	+	–	–	+	+	+	+
PECer	–	–	–	+	–	–	–	–	–	+	+	+	+
Glycosylated ceramides	–	–	–	+	–	–	–	–	–	–	–	+	+
ST	–	–	–	+	–	–	–	–	+	–	–	+	+
Glycerolipids													
TAG	–	–	–	–	–	–	–	–	+	–	+	+	+
Methodology	TLC and iodine staining		radiolabel and TLC	ESI-MSMS	FAB-MSMS	ESI-MSMS	ESI-MSMS	ESI-MSMS	ESI-MSMS	ESI-MSMS	ESI-MSMS	ESI-MS	**ESI-MSMS**
Resolution of molecular species	–	–	–	+	+	+	+	+	+	+	+	+	+
Quantification	–	–	–	–	+	+	+	+	+	+	+	+	+
Number of molecular species quantified	–	–	–	–	22	4	–	~22	400	~60	28	250	>500

TLC, thin layer chromatography; FAB-MSMS, fast atom bombardment tandem MS; +, achieved in the study; –, not achieved in the study. See also Figure S2.

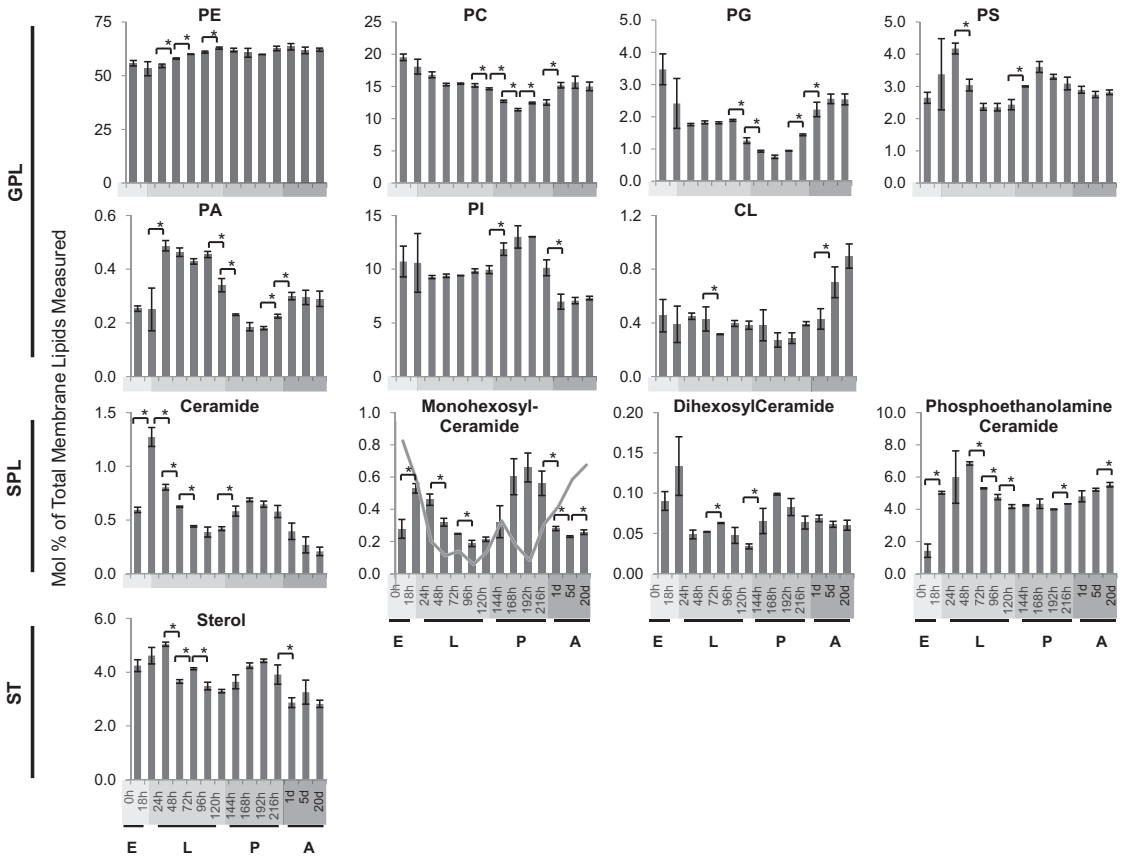


Figure 1. Temporal Changes in Membrane Lipid Classes during *Drosophila* Development

Twelve membrane lipid classes are analyzed in this study using multiple LC-MS/MS approaches. The data are a representative set (of three) containing duplicate samples per time point (n = 2). Mean values and average deviations are presented. Significance of difference between two adjacent time points is calculated by Student's t test, and *p < 0.05. Abbreviations: A, adult; CL, cardiolipin; E, embryo; L, larva; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol, PG, phosphatidylglycerol, PS, phosphatidylserine. P, pupa. The solid gray line in the HexCer panel represents the changes in expression level of *GlcT* involved in HexCer formation.
See also Figure S1 and Table S1.

Lipid components in eggs are largely determined by lipoproteins (Schonbaum et al., 1995); thus, we would expect high levels of SE in hemolymph of female flies. We next calculated the TAG/membrane lipid (GPL + SPL + ST) ratio as a rough proxy for the overall distribution of fatty acyl mass (Figure 3). As expected, the TAG/membrane lipid ratio rises sharply as the larvae grow and enter the pupal stage. However, it remains at this high level throughout all pupal stages, i.e., several days, in the absence of nutrient uptake. Together with the observation that the major membrane lipids are also largely unchanged in pupal stages (Figure 1), this suggests that TAG is not utilized as a source of energy via beta oxidation. Instead, the constant ratio might reflect a tight, albeit nonobvious, metabolic link of membrane to storage lipids that could be required for tissue remodeling during pupal development.

Stage-Dependent Variations in SPL Chemistry

A prominent feature of *Drosophila* lipids lies in the chemistry of its SPL, which are unique when compared with yeast and mammalian cells: (1) The sphingoid base is mainly d14 and d16, as opposed to d18 and d20, in mammalian and d/t18 and d/t20 in yeast [note, "d" and "t" stand for "di" and "tri" in this commonly used abbreviation to indicate the number of hydroxyls; thus, d18 and t18 refer to sphingoid bases (C18) with two and three hydroxyls, respectively (Chen et al., 2010)]; (2) sphingodienes, i.e., sphingoid bases with an additional double bond (for example, d16:2, Figure 4A); and (3) hydroxylated fatty acyls have been reported (for example, OH-22:0; Figure 4A; Fyrst et al., 2008; Carvalho et al., 2010). These characteristics add substantial chemical complexity that is likely to be biologically relevant (Guan et al., 2009; Hannun and Obeid, 2011; Merrill, 2011).

Figure 4 shows the relative proportions of the various forms of ceramides (Figure 4C), HexCer (Figure 4D), and PECer (Figure 4E) over the course of development. The main changes in HexCer during *Drosophila* development are the following: (1) a high relative abundance of d16 sphingoid base (approximately 50% of all HexCer contain d16; yellow shaded bars in Figure 4D) and hydroxylated fatty acyls during larval stages (striped bars in Figure 4D; (2) the dominance of d14 sphingoid base and nonhydroxylated fatty acyls in pupal stages (blue bars in Figure 4D); and (3) SPL with hydroxylated d14:1 ceramide backbones (light blue striped bars in Figures 4C and 4D) are largely restricted to

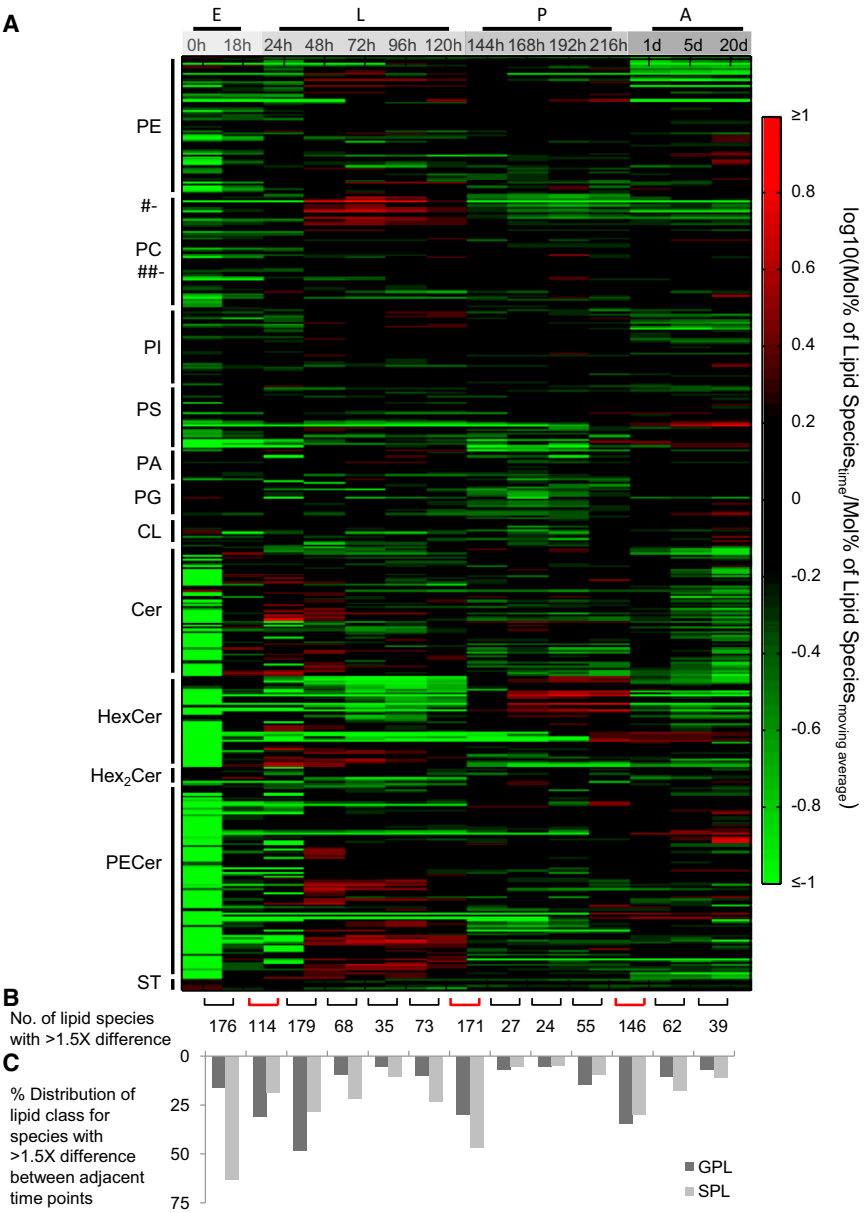


Figure 2. Survey Map for Relative Changes in Membrane Lipid Species between Developmental Stages

(A) Heatmap representation of changes of individual species of membrane lipids. Mole percent (Mol%) of individual lipid species was averaged across time to obtain the moving average value. The $\log_{10}$ ratio is then calculated for signals of each lipid species for each time point normalized to the moving average. #, cluster of PC species at larval stages that are much higher than the moving average; ##, cluster of PC species that are not changing temporally.

(B) Number of lipid species that are regulated at least 1.5-fold between two adjacent time points.

(C) Percentage of lipids species as a function of the total GPL or SPL detected that are regulated 1.5-fold between two adjacent time points. The entire detailed data set of this heatmap is listed in Table S2.

See also Figure S3.

HexCer in adult flies (Figure 4D) and are almost absent in other developmental stages (Figures 4C–4E). Note that (1) hexose identity and (2) the precise location of double bonds and hydroxylations cannot be derived from our analytical approach.

We propose that one possible way to mine such biochemical information is to correlate the lipidomic observations with gene expression analysis of characterized (and putative) lipid metabolic enzymes and their regulators. We curated the expression data reported by Graveley et al. for GC6437 that encodes glucosyltransferase (GlcT), an enzyme that catalyzes the formation of HexCer. The trend of *GlcT* expression is consistent with the overall levels of HexCer from early embryonic to early pupal stages but deviates in later developmental stages (Figure 1), suggesting that additional factors are required at later stages in development. Similary, fatty acid hydroxlase (*FA2H*/CG30502) is an SPL-specific hydroxylase in *Drosophila* (Carvalho et al., 2010), and its gene expression profile follows the observed trends in hydroxylated forms of SPL (e.g., HexCer) in larval stages but not in pupae and adult flies (Figure 4D).

Ceramides with d16 sphingoid bases are much more abundant in HexCer than in PECer (compare the sizes of yellow bars in Figures 4D and 4E). This probably reflects substantial selective substrate preference at the level of the ceramide precursor pool (Figure 4B). Therefore, the data presented in this study may represent a starting point for the characterization of SPL enzymes combining mass spectrometry with *Drosophila* genetics (Keays et al., 2011).

Gender-Specific Differences in Lipidome

Sexual maturation and high energy requirements are most obvious characteristics of the winged imago and both are expected to have profound effects on lipid metabolism. Phosphatidylglycerol (PG) and cardiolipin levels rise sharply during early adulthood, which is consistent with their abundance and roles in mitochondria (Figure 1). While we do not cover all lipids that have been described in sexual differentiation, oogenesis, and spermatogenesis (Terashima and Bownes, 2005), we applied our lipidomics approach to identify gender differences. Fatty acyl saturation has previously been described as a subtle gender bias in adult flies (Parisi et al., 2011). Consistent with that observation, we find elevated levels of lipid species with polyunsaturated fatty acyls (e.g., 38:4 forms of PC, PS, phosphatidylinositol [PI]) and concomitantly lowered levels of GPL with monounsaturated fatty acyls (Figure 5B), most prominently 40:1 PS (Figure S5) in 5- and 20-day-old females in comparison to males (Figure 5B). Another major difference between male and female flies

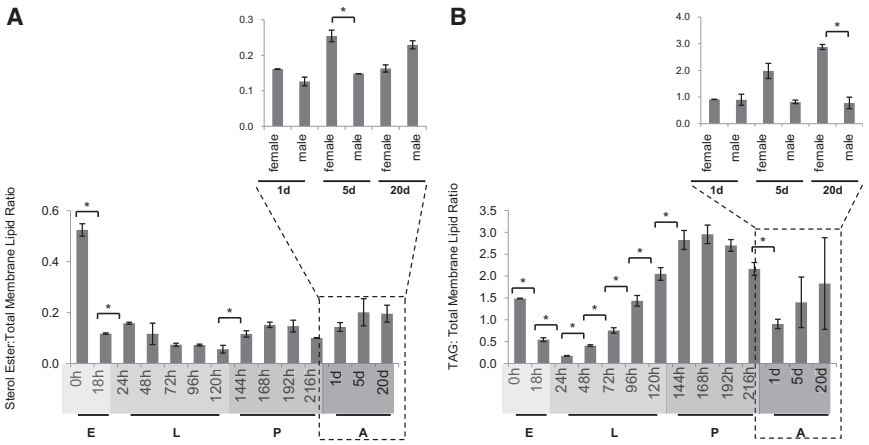


Figure 3. Stage- and Gender-Dependent Changes in Storage Lipids

(A) Sterol ester:total membrane lipid ratio during development.

(B) Triacylglycerol:total membrane ratio during development. Insets show the profiles of these lipids in adult female and male flies. The data are a representative set (out of three sets) containing duplicate samples per time point (n = 2). Mean values and average deviations are presented. Significance of difference between two adjacent time points and between male and female is calculated by Student's t test, and *p < 0.05.

throughout the first 20 days of adulthood is a profound inverse relationship in hydroxylation of PECer. Hydroxylated PECer are much more abundant in female flies, and nonhydroxylated forms are less abundant, compared to male flies of the same age (Figure 5B). Males, on the contrary, have more unsaturated fatty acids in their SPL (e.g., d14:1/24:1 PECer; Figure 5C), as well as odd chain fatty acyls (Figure S4). While not expected to make a strong contribution to lipid profiles (Parisi et al., 2011), we cannot exclude direct germline effects as gonads were included in the preparation procedure of adult flies.

ghiberti, a Gene Required for Male Meiotic Cytokinesis, Encodes a Protein Homologous to the Mammalian Small Subunit of Serine Palmitoyltransferase

We next applied our analytical tool for biochemical characterization of *ghiberti* (*ghi*; previously called *frodo*), which was isolated during a screen for genes required for meiotic cytokinesis in *Drosophila* males (Giansanti et al., 2004). Two mutant alleles were isolated: ghi^{z0287}, which causes cytokinesis failures in most meiotic divisions, and ghi^{z1497}, which disrupts cytokinesis in less than 20% of divisions. ghi^{z0287} mutant spermatocytes assemble a regular actomyosin ring, but the ring fails to constrict to completion and the furrow regresses, leading to cytokinesis failure (Giansanti et al., 2004). The same phenotype is caused by mutations in several genes that encode membrane traffic proteins, including the Cog5 subunit of the conserved oligomeric Golgi complex encoded by *four wheel stop* (*fws*) (Farkas et al., 2003); the TRAPPII subunit specified by *brunelleschi* (*bru*) (Robinett et al., 2009); the Rab11 and ARF6 GTPases (Dyer et al., 2007; Giansanti et al., 2007); the PI transfer protein encoded by *giotto* (*gio*) (Gatt and Glover, 2006; Giansanti et al., 2006); and the PI 4-kinase-beta (PI4Kbeta) encoded by *four wheel drive* (*fwd*) (Brill et al., 2000).

We cloned *ghi* by fine mapping followed by DNA sequencing, which showed that ghi^{z0287} carries an A→C transversion that changes the stop codon of *CG32038* into a tyrosine codon, leading to a 44 amino acid (aa) extension of the predicted 81 aa CG32038 protein. Transformation with a rescue construct and complementation experiments confirmed that *ghi* is indeed *CG32038* (see Experimental Procedures). *ghi* encodes a product that shares low homology with the small regulatory subunits of the serine palmitoyltransferase (ssSPTs) (Figure 6A), an enzyme that catalyzes the first step in SPL biosynthesis.

Using the methods described here, we found that the ghi^{z0287} mutation affects the d16/d14 ratio of SPLs in both third instar larvae (Figures 6B–6D) and adult testes (Figures 6E–6G). A shift toward increased formation of d16-containing SPL (Cer, HexCer, and PECer) was observed in ghi^{z0287}. Importantly, a similar trend was observed in testes, where SPL with the d16 sphingoid base are very low abundant in wild-type and dramatically elevated in the *ghi* mutant (Figures 6E–6G). Therefore, together with the (albeit) low sequence similarity to human ssSPT, this partial (biochemical) characterization provides strong experimental evidence for a role of *ghi* in early steps of SPL biosynthesis.

DISCUSSION

We document in a systematic fashion the levels of more than 500 (mainly membrane and some storage) lipids that are, to a large extent, identified species, i.e., chemical entities with a defined headgroup and hydrophobic chain configuration based on mass-to-charge (m/z) values in the mass spectrometric readout, although some values remain conflicted with anticipated isobaric overlap (particularly for PE and PC species, which harbor many ether-linked representatives; Table S2).

Several limitations of this resource that should be noted include (1) absolute quantification and lipidomic coverage, (2) variations that result from growth conditions and genetic background, and (3) spatial resolution. First, we acknowledge that matrix effects are not fully controlled for. Synthetic standards representing the 14 classes of lipids analyzed were used (see Experimental Procedures for the list of standards used; see Tables S1 and S2 for analytical conditions such as collision energies and transitions). However, it is difficult to judge how the properties of the different developmental stages (e.g., cuticular components, nonpolar-to-polar lipid ratios, water and salt content, etc.) will affect lipid recovery and analytical detection. Additional efforts will be required to include lipid mediators, e.g., SPL metabolites, which, as many genetic studies have shown, play critical roles in brain development and neuronal function in *Drosophila* (Kraut, 2011). Nonpolar compounds such as wax esters and hydrocarbons (important in cuticular and mating functions) may demand alternative extraction

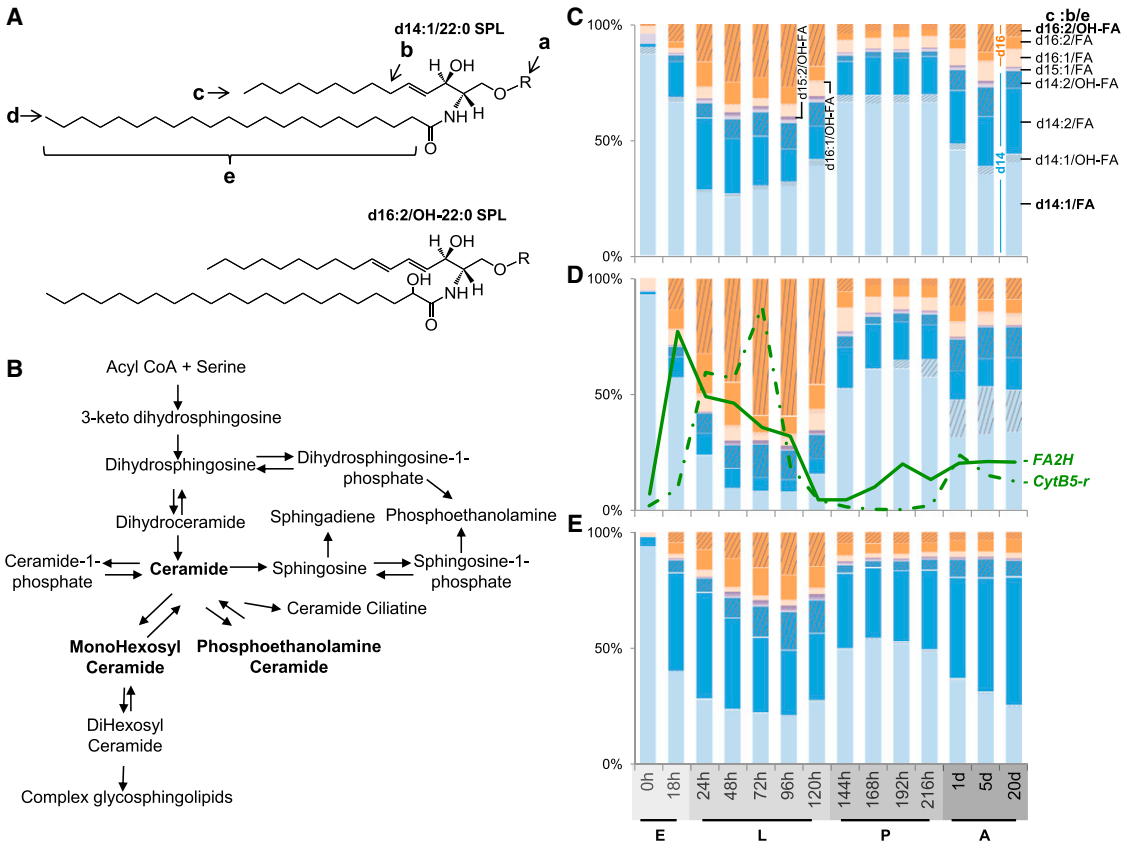


Figure 4. Changes in SPL Chemistries during *Drosophila* Development

(A) General structures of SPL and sites of modifications: (a) headgroup, (b) desaturation, (c) sphingoid base chain length, (d) fatty acyl chain length, and (e) fatty acyl hydroxylation (-OH). Resolution of these chemical details is achieved in this study by multiple LC-MS/MS approaches.

(B) Scheme of SPL metabolism in *D. melanogaster*.

(C) Temporal changes in sphingoid base chemistry and fatty acyl hydroxylation of ceramides.

(D) Temporal changes in sphingoid base chemistry and fatty acyl hydroxylation of HexCer. E. Temporal changes in sphingoid base chemistry and fatty acyl hydroxylation of PECer. Genes responsible for SPL desaturation/hydroxylations (*FA2H*/CG30502 and *Cyt-b5-r*/CG13279) are curated from FlyBase (Graveley et al., 2011) and superimposed with the changes in lipid profiles. Abbreviations: FA, fatty acyl; OH-FA, hydroxylated fatty acyls.

See also Figure S4.

protocols as well as different analytical conditions such as atmospheric pressure chemical ionization (APCI). Second, it is well known that lipid compositions in *Drosophila* are highly dependent on the diet, temperature, and light conditions (Stark et al., 1993; Overgaard et al., 2005; Carvalho et al., 2010), but understanding these variables was not the major aim of this study. Instead, the effect of diet of *Drosophila* lipidomes were recently reported in a study using shotgun lipidomics, i.e., direct profiling of lipids in complex mixtures without the use of LC (Carvalho et al., 2012). Third, as we chose to use extracts of entire organisms (rather than dissected organs, except for SPL contents of testis, Figures 6E–6G), information on tissue distribution of lipids (Carvalho et al., 2012) is not resolved in this study. Tissue imaging via MS, an upcoming new discipline in lipidomics (Sullards et al., 2011; Murphy and Merrill, 2011) will therefore be complementary to our description to provide spatial information. Such approaches, together with turnover experiments using organs rather than whole organisms, will be very powerful future avenues to study the relationships of (diffusible) lipid mediators in connection to expression of bona fide lipid enzymes and regulators of lipid flux (Yuan et al., 2011) including cell-nonautomomous enzymes (Acharya et al., 2008).

In addition to a technical resource, this report represents a biochemical resource for research in *Drosophila* development. The entire data set, i.e., values for all lipids across all time points, is provided in Table S2. Furthermore, the ~200 SPLs reported here have been deposited into the LIPID MAPS structural database, where they are publicly available (http://www.lipidmaps.org; see also Table S2, LipidMaps ID).

Our study revealed particularly interesting insights on SPL diversity and biochemistry during fly development as well as gender differences. In *Drosophila*, the desaturase gene family exhibits sex-specific expression (Keays et al., 2011). For instance, Des1 (*ifc1*/ CG9078) is a sphingolipid delta-4 desaturase (Ternes et al., 2002) required for spermatogenesis but not oogenesis (Castrillon et al., 1993). Sxe1, a member of the cytochrome P450 enzyme family, affects fatty acyl metabolism and is required for efficient male mating, although its precise enzymatic activities remain to be determined (Fujii et al., 2008). Our results shown in Figure 5 provide experimental evidence for

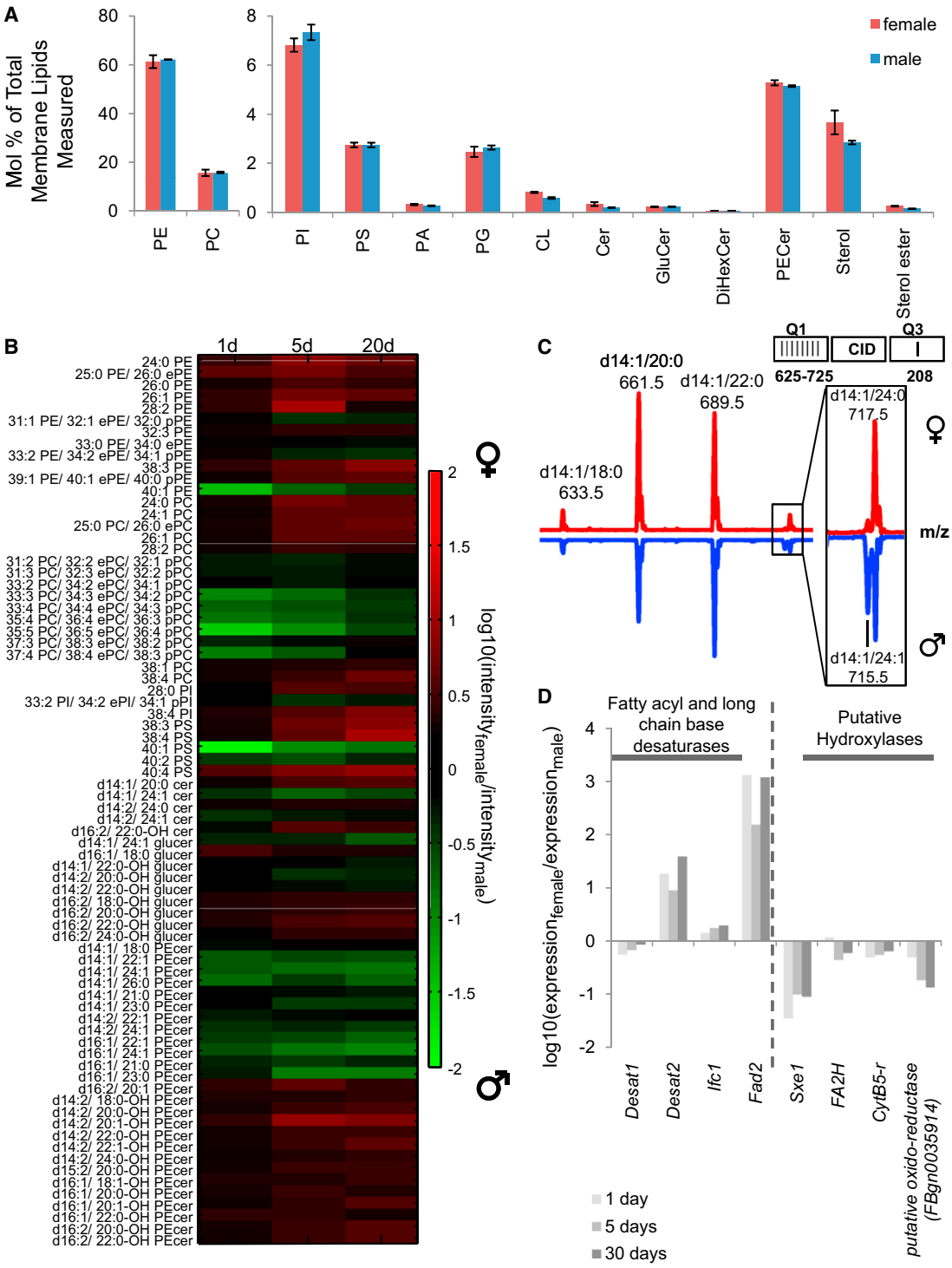


Figure 5. Gender-Specific Differences in Lipid Composition

(A) Lipid classes distribution between adult female and male flies (5 days old). Mean values and average deviations are presented.

(B) Heatmap representation of lipid species that are regulated at least 1.5-fold between males and females. The data are a representative set (out of three) containing duplicate samples. Mean values are obtained for the mole percent of individual lipids species, and the $\log_{10}$ ratio of the values for female to males are then computed.

(C) Precursor ion scans profiles of d14:1-containing PECer reveal (and confirm) an increase in fatty acid desaturation in SPL of male adult flies.

(D) Curation of genes responsible for lipid desaturation/hydroxylations in adult flies (FlyBase; Graveley et al., 2011). Abbreviations: ePC, ether PC; pPC, plasmalogen PC; ePE, ether PE; pPE, plasmalogen PE.

See also Figure S5.

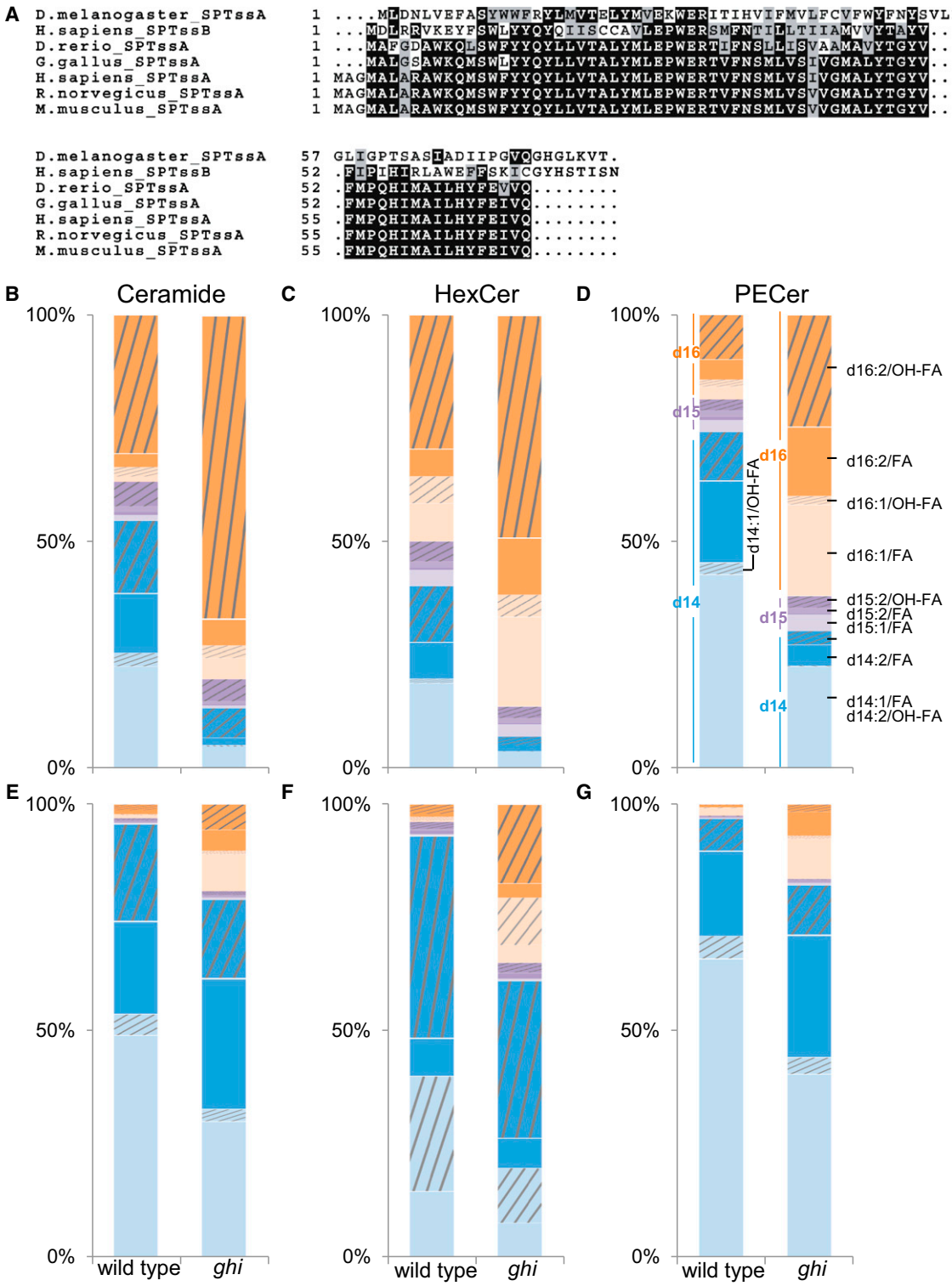


Figure 6. *ghiberti* Is a Modulator of SPL and Homolog of Mammalian ssSPT

(A) Homology alignment of ssSPT amino acid sequences. The aa sequences of human ssSPTA and ssSPTB, rat ssSPTA, mouse ssSPTA, chicken ssSPTA, the closest homolog of ssSPTA in zebrafish, and fly ssSPTA (*ghiberti*) are aligned. Note that the major conservation among human ssSPTA and ssSPTB is concentrated in the central region proximal to the NH terminus, which is also the only region quite conserved in *Drosophila* ssSPTA. Identical residues are shaded with black boxes, and light gray boxes indicate similar residues. Multiple protein alignment was done with online tool MUltiple (S)equence (C)omparison by (L)og-(E)xpectation (EMBL-EBI) and shading using the online Boxshade program.

(B) Sphingoid base chemistry and fatty acyl hydroxylation of ceramides of wild-type and *ghi* third instar larvae.

(legend continued on next page)

a putative role of PECer (as opposed to other SPL) in such gender-specific effects.

Hydroxylated PECer are highly enriched in female flies, which raises the possibility that they play a role in reproductive processes such as egg production. Hydroxylated forms of diene PECer are highly specific in the gut, whereas other PECer are also present in the fat body or brain (Carvalho et al., 2010). The precise cellular localization and possible contribution to barrier functions (Hama, 2010), e.g., via intermolecular hydrogen bonding (Lundén et al., 1977), in epithelial layers (Sampaio et al., 2011) remain to be determined. The gender differences in hydroxylated PECer reported here may affect the uptake of nutrients including sterols, which is particularly relevant since *Drosophila* is a sterol auxotroph and is therefore dependent on dietary sterols (Carvalho et al., 2012). In female adults, this could be critical for egg development, since sterol esters are enriched in early embryos (Figure 3A). The gender differences reported here will furthermore be of interest to studies that aim at understanding the fundamentals of chemical communication underlying courtship where lipids have been shown to play a key role in regulating attractiveness during mating (Yew et al., 2009).

The biochemical information as described here will help future studies investigating the regulation of membrane lipid homeostasis. For example, in mammals, processing of ST regulatory element-binding protein (SREBP) is controlled largely by sterols (Goldstein et al., 2006), whereas PE lipids seem to be key regulators in *Drosophila* (Dobrosotskaya et al., 2002). In nematodes (*Caenorhabditis elegans*), a circuit between PC and SREBP regulates lipogenesis (Walker et al., 2011). The study of SPL metabolism in very early biosynthetic events where amino acid and fatty acid metabolism merge (Pruett et al., 2008; Breslow et al., 2010) or the characterization of mutations that affect this stage (Momin et al., 2009) are other examples.

SPT is involved in the first step of SPL biosynthesis. The subunits of the heteromeric enyzme are highly regulated, and mutations of this enzyme have been implicated in human hereditary sensory neuropathy type I disease (Dawkins et al., 2001; Bejaoui et al., 2002), in part due to generation of 1-deoxysphinganine, a novel sphingoid base (Gable et al., 2010; Penno et al., 2010). Understanding the precise details that govern substrate recognition and specificity (Han et al., 2009) and control of SPL homeostasis overall (Breslow et al., 2010) are therefore very intense areas of current research. The difficulty to predict SPT regulators solely on sequence homology between organisms has been proven to be one critical challenge in such endeavors. Here, we combined genetics with the technical resource for identification and partial characterization of a SPT regulator in *Drosophila melanogaster* uncovered in this study. Second, the finding that mutations in *ghi* cause a drastic change d14/d16 sphingoid bases ratio provides a possible explanation for the cytokinesis defect caused by *ghi* mutations. One can envisage that the altered d14/d16 balance observed in *ghi* mutant testes would affect membrane structure and physical properties, impairing the very specialized membrane-related events essential for cytokinesis. However, given the likely role of *ghi* in the very initial stages of SPL biosynthesis, our current results do not exclude that other downstream effects of *ghi* mutations would affect cytokinesis. For example, *ghi* may disrupt cytokinesis by affecting the levels and/or the hydroxylation patterns of many SPLs (PECer, Hex-Cer, soluble sphingoid base mediators, etc.). Third, *ghi* and related modulators of SPL might play more general roles as molecular links between membrane homeostasis and cellular metabolism (Roelants et al., 2011).

It will be interesting to include and evaluate the effects of other (related) lipid enzymes in spermatogenesis (Wang and Huang, 2012), such as *bond*, a fatty acyl elongase required for spermatocyte cytokinesis (Szafer-Glusman et al., 2008) that could lead to chain length modifications in glycerophospholipids and sphingolipids and therefore might act in concert with *ghi.* Taken together, the combination of the biochemical approach described here with complementary genetics-driven systems biology in *Drosophila* (Pospisilik et al., 2010) is a promising avenue to yield valuable insights into the role of lipids in biological systems.

In summary, we report the development of a lipidomics tool for probing lipid functions in *Drosophila melanogaster.* The relevance and importance of our work is further emphasized by a related study that was published during manuscript revision for this article (Carvalho et al., 2012). Common to both studies is the use of MS, albeit with some notable differences. We used liquid chromatography-multiple reaction monitoring (MRM) and focused primarily on membrane lipids with coverage of >500 lipid species and a particular emphasis on SPL (which alone covered 210 species). Carvalho et al. (2012) instead covered 250 lipids species by direct infusion (i.e., without the use of LC) high resolution MS. While detailed direct comparison is difficult due to differences in lipid coverage and data normalization, both studies report similar trends (e.g., in TAG/membrane lipid time course). Another major difference between the two studies clearly lies in the applications of the lipidomics tools. We illustrated how to use our informational resource for data mining (within the lipidomics data set as well as in comparison with publicly available gene expression studies) and as a technical tool for characterization of a modulator of SPL identified by genetics. Carvalho et al. (2012) carefully studied the variations of tissue lipidomes as a function of different diets in one laboratory strain. Therefore, it can be expected that these two reference works will be highly complementary.

EXPERIMENTAL PROCEDURES

Analytical Reagents

All organic solvents and additives were of LC or LC-MS grade and were obtained from Sigma-Adrich. Other chemicals were of analytical grades.

(C) Sphingoid base chemistry and fatty acyl hydroxylation of HexCer of wild-type and *ghi* third instar larvae.
(D) Sphingoid base chemistry and fatty acyl hydroxylation of PECer of wild-type and *ghi* third instar larvae.
(E) Sphingoid base chemistry and fatty acyl hydroxylation of ceramides of wild-type and *ghi* adult testes.
(F) Sphingoid base chemistry and fatty acyl hydroxylation of HexCer of wild-type and *ghi* adult testes.
(G) Sphingoid base chemistry and fatty acyl hydroxylation of PECer of wild-type and *ghi* adult testes. The data are represented as the mean values from three independent experimental preparations per genotype (n = 3 with two larvae and 50 testes per condition).

Drosophila Sample Collection

All developmental samples were prepared using the Oregon R wild-type strain and were reared on a standard yeast-based medium (for 15 l: 90 g Agar, 525 g yeast, 1,200 g corn flour, 1,050 g sugar, 30 ml nipagine solution [15% in ethanol], 150 ml propionic acid) at 25°C. Embryos were collected as 6 hr egg lays, which were allowed to develop to the desired intervals. At least two prelays of 1 hr were performed before each collection.

For embryonic stages, samples were collected at 0 hr and 18 hr after egg laying (AEL) and washed three times with PBS to remove debris and yeast. The embryos were then dechorionated in 20% bleach and washed three times with PBS before snap freezing in liquid nitrogen. Typically, 30 embryos were used for each time point.

Larvae and pupae were raised from 6 hr embryonic egg lays and collected at 24 hr intervals, starting at 24 hr after egg laying. In total, five larval (24, 28, 72, 96, and 120 hr AEL) and four pupal (144, 168, 192, and 216 hr AEL) collections were taken. Typically, 25 larvae were collected per replicate for the early stages (24 and 48 hr), five larvae for 72 hr AEL, and two larvae or pupae for the later stages (96–216 hr). Larvae and pupae were washed in PBS to remove debris and yeast and snap frozen in liquid nitrogen.

Adult flies were synchronized by collection within the first 6 hr of eclosion and allowed to develop for 1, 5, and 20 days. The adults were harvested by shaking into tubes and immediately snap frozen in liquid nitrogen. The animals were then sorted by gender on a plastic sheet over dry ice. Two adults were collected for each replicate. The animals were rinsed with PBS prior to lipid extraction. Each series of experiments comprised at least duplicates for each condition, and the time course was repeated three times with a different group of parents raised separately.

Cloning of *ghi*

ghiberti (*ghi*; previously called *frodo*) is an ethyl methane sulfonate-induced, male sterile mutation uncovered by the 1Mb deficiency *Df(3L)AC1* that removes region 67A2-D13 (Giansanti et al., 2004). To restrict *ghi* map position, we performed Delta 2-3 transposase-induced P element-mediated male recombination (Chen et al., 1998). Examination of recombinant males for male fertility placed *ghi* between the P element insertions EP3710 and EP559. Using bulk PCR products, we then sequenced the 10 kb interval between the two P element insertion sites in both *ghi/Df(3L)AC1* and the wild-type isogenic stock used for mutagenesis. We found that ghi^{z0287} carries an A→C transversion that changes the stop codon of *CG32038* to a tyrosine codon adding an additional 44 aa stretch to the predicted CG32038 protein of 81 aa. To confirm that *CG32038* is *ghi*, we generated a genomic rescue construct of 1.7 kb by PCR using the 5′AGCGGCCGCgaacgctggtttttcgaca tcttcc3′ and 5′AGCGGCCGCtagagcattgaagtacggatttagc3′ primers, both containing a NotI site. This construct contains less than half of the *CG3448* coding sequence (the gene upstream of *CG32038*) and none of the coding sequence of *CG3445/phol* (the gene downstream of *CG32038*). The rescue construct was then inserted in pCaSpeR4 using the NotI sites and injected in embryos for germline transformation. Three insertions on the second chromosome were recovered, and each was able to rescue the meiotic cytokinesis defect in *ghi* mutant males. Lipid extracts were prepared from two third instar larvae and 50 testes dissected from adult males, per genotype per sample.

Lipid Standards

We obtained 34:1 D31-PA, 34:1 D31-PC, 34:1 D31-PE, 34:1 D31-PI, 34:1 D31-PS, 34:1 D31-PG, 28:0 PC, 28:0 PE, 28:0 PS, 12:0/d17:1 PE-Ceramide, 8:0/d18:1 Glucosylceramide, 8:0/d18:1 Lactosylceramide, 17:0/d18:1 Ceramide, Tetramyristoyl cardiolipin from Avanti Polar Lipids (Alabaster, AL, USA). Cholesterol-D6, Cholesterol-D6-stearate, and 48:0 D5-TAG were obtained from CDN Isotopes Inc. (Pointe-Claire, Quebec, Canada). Dioctanoyl PI was purchased from Echelon Biosciences, Inc. (Salt Lake City, UT, USA).

Lipid Extraction

Samples were homogenized in 100 μl of PBS using a handheld motorized pestle, and lipids were extracted by modified Bligh and Dyer's method (Bligh and Dyer, 1959). Briefly, 600 μl of chloroform:methanol (1:2, v/v) was added to the homogenate, and the mixture was vortexed for 1 min. The samples were placed on a thermomixer and shaken at 1,000 rpm for 2 hr at 4°C. We added 200 μl of chloroform and 250 μl of water to the mixture, which was vortexed for 1 min. The phases were separated by centrifugation at 9,000 rpm for 2 min, and the lower organic phase was collected. We added 400 μl of chloroform to the aqueous phase, and phase separation was repeated. The two organic extracts were pooled and concentrated by drying under vacuum using a Centrivap (Labconco Corporation, Kansas City, MO, USA). The dried lipid film was resuspended in 100 μl of chloroform-methanol (1:1, v/v). These lipid extracts were spiked with internal standards and analyzed using LC-MS as described previously (Shui et al., 2010a, 2010b).

Analysis of Lipids using LC-MS

A range of different platforms were used for qualitative (Thermo Orbitrap) and semiquantitative (ABI 4000QT and Agilent 6460) analyses. Results between these platforms are comparable once analytical instrument conditions are optimized. Here, we provide the analytical parameters for one of the systems, namely, a high-performance liquid chromatography system (Agilent 1260) coupled with a triple quadrupole mass spectrometer (Agilent 6460), which was used for characterization and quantification of lipids. GPL and SPL were separated using normal phase chromatography (Shui et al., 2010a), while neutral lipids (TAG and ST) were separated using reverse phase chromatography as described previously (Shui et al., 2010b). The MS conditions were as follows. For ESI: gas temperature, 300°C; gas flow, 10 l/min; sheath gas temperature, 350°C; sheath gas flow, 8 l/min; and capillary voltage, 3,500 V. For APCI: gas temperature, 300°C; vaporizer, 450°C; gas flow, 5 l/min; capillary voltage, 4,000 V; and corona current, 4 μA.

Precursor ion or neutral loss scans of GPL headgroup fragments were used to obtain information on *Drosophila* GPL compositions, while SPL were resolved using precursor scans of dehydrated sphingoid bases (Table S1) (Sullards and Merrill, 2001; Han and Gross, 2005). Based on this information, a list of MRM transitions was set up to follow GPL and SPL (Tables S1 and S2). For ST (ergosterol, cholesterol, and desmosterol) and their esters, TAG analyses were performed using MRM and selected ion monitoring, respectively. Each individual ion dissociation pathway was optimized with regard to collison energy and fragmentor voltage to minimize variations in relative ion abundance due to differences in dissociations (Sullards and Merrill, 2001; Merrill et al., 2005).

Data processing, including peak smoothing and integration of areas under the curves for each ions measured, was performed using the MassHunter Quantification Software (Agilent). The final representations of the data were obtained through further computation using the following formulae:

$$\text{Mol\% of lipid species 1 as a function of total membrane lipids} = \frac{\text{lipid 1 (mol)}}{\text{sum of all membrane lipid species (mol)}}$$

$$\text{Mol\% of lipid class as a function of total membrane lipids} = \frac{\text{sum of lipid species of a class (e.g., all PA species, in mol)}}{\text{sum of all membrane lipid species (mol)}}$$

$$\text{Mol\% moving average of lipid species 1} = \text{average mol\% of lipid species 1 across time}$$

$$\text{Relative deviation from moving average} = \log_{10} \text{ratio}\left(\frac{\text{mol\% time}}{\text{mol\% moving average}}\right)$$

Comparisons of the means of lipids were performed between developmental times, as well as between male and female flies. Data are expressed as means with average deviations as indicated by the error bars (Table S2). The differences between developmental times as well as for genders were determined statistically using Student's t test, and $p < 0.05$ was considered statistically significant.

SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.devcel.2012.11.012.

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REFERENCES

Acharya, U., and Acharya, J.K. (2005). Enzymes of sphingolipid metabolism in Drosophila melanogaster. Cell. Mol. Life Sci. *62*, 128–142.

Acharya, J.K., Dasgupta, U., Rawat, S.S., Yuan, C., Sanxaridis, P.D., Yonamine, I., Karim, P., Nagashima, K., Brodsky, M.H., Tsunoda, S., and Acharya, U. (2008). Cell-nonautonomous function of ceramidase in photoreceptor homeostasis. Neuron *57*, 69–79.

Bartke, N., and Hannun, Y.A. (2009). Bioactive sphingolipids: metabolism and function. J. Lipid Res. Suppl. *50*, S91–S96.

Bauer, R. (2010). Towards understanding regulation of energy homeostasis by ceramide synthases. Results Probl. Cell Differ. *52*, 175–181.

Becker, G.W., and Lester, R.L. (1980). Biosynthesis of phosphoinositol-containing sphingolipids from phosphatidylinositol by a membrane preparation from Saccharomyces cerevisiae. J. Bacteriol. *142*, 747–754.

Bejaoui, K., Uchida, Y., Yasuda, S., Ho, M., Nishijima, M., Brown, R.H., Jr., Holleran, W.M., and Hanada, K. (2002). Hereditary sensory neuropathy type 1 mutations confer dominant negative effects on serine palmitoyltransferase, critical for sphingolipid synthesis. J. Clin. Invest. *110*, 1301–1308.

Bithell, A., Finch, S.E., Hornby, M.F., and Williams, B.P. (2008). Fibroblast growth factor 2 maintains the neurogenic capacity of embryonic neural progenitor cells in vitro but changes their neuronal subtype specification. Stem Cells *26*, 1565–1574.

Bligh, E.G., and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. *37*, 911–917.

Breslow, D.K., Collins, S.R., Bodenmiller, B., Aebersold, R., Simons, K., Shevchenko, A., Ejsing, C.S., and Weissman, J.S. (2010). Orm family proteins mediate sphingolipid homeostasis. Nature *463*, 1048–1053.

Brill, J.A., Hime, G.R., Scharer-Schuksz, M., and Fuller, M.T. (2000). A phospholipid kinase regulates actin organization and intercellular bridge formation during germline cytokinesis. Development *127*, 3855–3864.

Carvalho, M., Schwudke, D., Sampaio, J.L., Palm, W., Riezman, I., Dey, G., Gupta, G.D., Mayor, S., Riezman, H., Shevchenko, A., et al. (2010). Survival strategies of a sterol auxotroph. Development *137*, 3675–3685.

Carvalho, M., Sampaio, J.L., Palm, W., Brankatschk, M., Eaton, S., and Shevchenko, A. (2012). Effects of diet and development on the Drosophila lipidome. Mol. Syst. Biol. *8*, 600.

Castrillon, D.H., Gönczy, P., Alexander, S., Rawson, R., Eberhart, C.G., Viswanathan, S., DiNardo, S., and Wasserman, S.A. (1993). Toward a molecular genetic analysis of spermatogenesis in Drosophila melanogaster: characterization of male-sterile mutants generated by single P element mutagenesis. Genetics *135*, 489–505.

Chen, B., Chu, T., Harms, E., Gergen, J.P., and Strickland, S. (1998). Mapping of Drosophila mutations using site-specific male recombination. Genetics *149*, 157–163.

Chen, Y., Liu, Y., Sullards, M.C., and Merrill, A.H., Jr. (2010). An introduction to sphingolipid metabolism and analysis by new technologies. Neuromolecular Med. *12*, 306–319.

Chen, Y.W., Pedersen, J.W., Wandall, H.H., Levery, S.B., Pizette, S., Clausen, H., and Cohen, S.M. (2007). Glycosphingolipids with extended sugar chain have specialized functions in development and behavior of Drosophila. Dev. Biol. *306*, 736–749.

Contreras, F.X., Ernst, A.M., Haberkant, P., Björkholm, P., Lindahl, E., Gönen, B., Tischer, C., Elofsson, A., von Heijne, G., Thiele, C., et al. (2012). Molecular recognition of a single sphingolipid species by a protein's transmembrane domain. Nature *481*, 525–529.

Dasgupta, U., Bamba, T., Chiantia, S., Karim, P., Tayoun, A.N., Yonamine, I., Rawat, S.S., Rao, R.P., Nagashima, K., Fukusaki, E., et al. (2009). Ceramide kinase regulates phospholipase C and phosphatidylinositol 4, 5, bisphosphate in phototransduction. Proc. Natl. Acad. Sci. USA *106*, 20063–20068.

Dawkins, J.L., Hulme, D.J., Brahmbhatt, S.B., Auer-Grumbach, M., and Nicholson, G.A. (2001). Mutations in SPTLC1, encoding serine palmitoyltransferase, long chain base subunit-1, cause hereditary sensory neuropathy type I. Nat. Genet. *27*, 309–312.

Dennis, E.A., Deems, R.A., Harkewicz, R., Quehenberger, O., Brown, H.A., Milne, S.B., Myers, D.S., Glass, C.K., Hardiman, G., Reichart, D., et al. (2010). A mouse macrophage lipidome. J. Biol. Chem. *285*, 39976–39985.

Dobrosotskaya, I.Y., Seegmiller, A.C., Brown, M.S., Goldstein, J.L., and Rawson, R.B. (2002). Regulation of SREBP processing and membrane lipid production by phospholipids in Drosophila. Science *296*, 879–883.

Dyer, N., Rebollo, E., Domínguez, P., Elkhatib, N., Chavrier, P., Daviet, L., González, C., and González-Gaitán, M. (2007). Spermatocyte cytokinesis requires rapid membrane addition mediated by ARF6 on central spindle recycling endosomes. Development *134*, 4437–4447.

Ejsing, C.S., Sampaio, J.L., Surendranath, V., Duchoslav, E., Ekroos, K., Klemm, R.W., Simons, K., and Shevchenko, A. (2009). Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. Proc. Natl. Acad. Sci. USA *106*, 2136–2141.

Farkas, R.M., Giansanti, M.G., Gatti, M., and Fuller, M.T. (2003). The Drosophila Cog5 homologue is required for cytokinesis, cell elongation, and assembly of specialized Golgi architecture during spermatogenesis. Mol. Biol. Cell *14*, 190–200.

Fujii, S., Toyama, A., and Amrein, H. (2008). A male-specific fatty acid omega-hydroxylase, SXE1, is necessary for efficient male mating in Drosophila melanogaster. Genetics *180*, 179–190.

Fyrst, H., Herr, D.R., Harris, G.L., and Saba, J.D. (2004). Characterization of free endogenous C14 and C16 sphingoid bases from Drosophila melanogaster. J. Lipid Res. *45*, 54–62.

Fyrst, H., Zhang, X., Herr, D.R., Byun, H.S., Bittman, R., Phan, V.H., Harris, G.L., and Saba, J.D. (2008). Identification and characterization by electrospray mass spectrometry of endogenous Drosophila sphingadienes. J. Lipid Res. *49*, 597–606.

Gable, K., Gupta, S.D., Han, G., Niranjanakumari, S., Harmon, J.M., and Dunn, T.M. (2010). A disease-causing mutation in the active site of serine palmitoyltransferase causes catalytic promiscuity. J. Biol. Chem. *285*, 22846–22852.

Gamo, S., Kawabe, A., Kohara, H., Yamaguchi, H., Tanaka, Y., and Yagi, S. (1999). Fast atom bombardment tandem mass spectrometric analysis of phospholipids in Drosophila melanogaster. J. Mass Spectrom. *34*, 590–600.

Gatt, M.K., and Glover, D.M. (2006). The Drosophila phosphatidylinositol transfer protein encoded by vibrator is essential to maintain cleavage-furrow ingression in cytokinesis. J. Cell Sci. *119*, 2225–2235.

Giansanti, M.G., Farkas, R.M., Bonaccorsi, S., Lindsley, D.L., Wakimoto, B.T., Fuller, M.T., and Gatti, M. (2004). Genetic dissection of meiotic cytokinesis in Drosophila males. Mol. Biol. Cell *15*, 2509–2522.

Giansanti, M.G., Bonaccorsi, S., Kurek, R., Farkas, R.M., Dimitri, P., Fuller, M.T., and Gatti, M. (2006). The class I PITP giotto is required for Drosophila cytokinesis. Curr. Biol. *16*, 195–201.

Giansanti, M.G., Belloni, G., and Gatti, M. (2007). Rab11 is required for membrane trafficking and actomyosin ring constriction in meiotic cytokinesis of Drosophila males. Mol. Biol. Cell *18*, 5034–5047.

Goldstein, J.L., DeBose-Boyd, R.A., and Brown, M.S. (2006). Protein sensors for membrane sterols. Cell *124*, 35–46.

Graveley, B.R., Brooks, A.N., Carlson, J.W., Duff, M.O., Landolin, J.M., Yang, L., Artieri, C.G., van Baren, M.J., Boley, N., Booth, B.W., et al. (2011). The developmental transcriptome of Drosophila melanogaster. Nature *471*, 473–479.

Guan, X.L., Souza, C.M., Pichler, H., Dewhurst, G., Schaad, O., Kajiwara, K., Wakabayashi, H., Ivanova, T., Castillon, G.A., Piccolis, M., et al. (2009). Functional interactions between sphingolipids and sterols in biological membranes regulating cell physiology. Mol. Biol. Cell *20*, 2083–2095.

Hama, H. (2010). Fatty acid 2-Hydroxylation in mammalian sphingolipid biology. Biochim. Biophys. Acta *1801*, 405–414.

Hammad, L.A., Cooper, B.S., Fisher, N.P., Montooth, K.L., and Karty, J.A. (2011). Profiling and quantification of Drosophila melanogaster lipids using liquid chromatography/mass spectrometry. Rapid Commun. Mass Spectrom. *25*, 2959–2968.

Han, G., Gupta, S.D., Gable, K., Niranjanakumari, S., Moitra, P., Eichler, F., Brown, R.H., Jr., Harmon, J.M., and Dunn, T.M. (2009). Identification of small subunits of mammalian serine palmitoyltransferase that confer distinct acyl-CoA substrate specificities. Proc. Natl. Acad. Sci. USA *106*, 8186–8191.

Han, X., and Gross, R.W. (2005). Shotgun lipidomics: multidimensional MS analysis of cellular lipidomes. Expert Rev. Proteomics *2*, 253–264.

Hannich, J.T., Umebayashi, K., and Riezman, H. (2011). Distribution and functions of sterols and sphingolipids. Cold Spring Harb. Perspect. Biol. *3*, 3.

Hannun, Y.A., and Obeid, L.M. (2011). Many ceramides. J. Biol. Chem. *286*, 27855–27862.

Jones, H.E., Harwood, J.L., Bowen, I.D., and Griffiths, G. (1992). Lipid composition of subcellular membranes from larvae and prepupae of Drosophila melanogaster. Lipids *27*, 984–987.

Keays, M.C., Barker, D., Wicker-Thomas, C., and Ritchie, M.G. (2011). Signatures of selection and sex-specific expression variation of a novel duplicate during the evolution of the Drosophila desaturase gene family. Mol. Ecol. *20*, 3617–3630.

Kohyama-Koganeya, A., Nabetani, T., Miura, M., and Hirabayashi, Y. (2011). Glucosylceramide synthase in the fat body controls energy metabolism in Drosophila. J. Lipid Res. *52*, 1392–1399.

Kraut, R. (2011). Roles of sphingolipids in Drosophila development and disease. J. Neurochem. *116*, 764–778.

Lingwood, D., Binnington, B., Róg, T., Vattulainen, I., Grzybek, M., Coskun, U., Lingwood, C.A., and Simons, K. (2011). Cholesterol modulates glycolipid conformation and receptor activity. Nat. Chem. Biol. *7*, 260–262.

Lundén, B.M., Löfgren, H., and Pascher, I. (1977). Accommodation of hydroxyl groups and their hydrogen bond system in a hydrocarbon matrix. Chem. Phys. Lipids *20*, 263–271.

Masood, M.A., Yuan, C., Acharya, J.K., Veenstra, T.D., and Blonder, J. (2010). Quantitation of ceramide phosphorylethanolamines containing saturated and unsaturated sphingoid base cores. Anal. Biochem. *400*, 259–269.

Merrill, A.H., Jr. (2011). Sphingolipid and glycosphingolipid metabolic pathways in the era of sphingolipidomics. Chem. Rev. *111*, 6387–6422.

Merrill, A.H., Jr., Sullards, M.C., Allegood, J.C., Kelly, S., and Wang, E. (2005). Sphingolipidomics: high-throughput, structure-specific, and quantitative analysis of sphingolipids by liquid chromatography tandem mass spectrometry. Methods *36*, 207–224.

Momin, A.A., Park, H., Allegood, J.C., Leipelt, M., Kelly, S.L., Merrill, A.H., Jr., and Hanada, K. (2009). Characterization of mutant serine palmitoyltransferase 1 in LY-B cells. Lipids *44*, 725–732.

Murphy, R.C., and Merrill, A.H., Jr. (2011). Lipidomics and imaging mass spectrometry. Biochim. Biophys. Acta *1811*, 635–636.

Overgaard, J., Sørensen, J.G., Petersen, S.O., Loeschcke, V., and Holmstrup, M. (2005). Changes in membrane lipid composition following rapid cold hardening in Drosophila melanogaster. J. Insect Physiol. *51*, 1173–1182.

Parisi, M., Li, R., and Oliver, B. (2011). Lipid profiles of female and male Drosophila. BMC. Res. Notes *4*, 198.

Penno, A., Reilly, M.M., Houlden, H., Laurá, M., Rentsch, K., Niederkofler, V., Stoeckli, E.T., Nicholson, G., Eichler, F., Brown, R.H., Jr., et al. (2010). Hereditary sensory neuropathy type 1 is caused by the accumulation of two neurotoxic sphingolipids. J. Biol. Chem. *285*, 11178–11187.

Pospisilik, J.A., Schramek, D., Schnidar, H., Cronin, S.J., Nehme, N.T., Zhang, X., Knauf, C., Cani, P.D., Aumayr, K., Todoric, J., et al. (2010). Drosophila genome-wide obesity screen reveals hedgehog as a determinant of brown versus white adipose cell fate. Cell *140*, 148–160.

Pruett, S.T., Bushnev, A., Hagedorn, K., Adiga, M., Haynes, C.A., Sullards, M.C., Liotta, D.C., and Merrill, A.H., Jr. (2008). Biodiversity of sphingoid bases ("sphingosines") and related amino alcohols. J. Lipid Res. *49*, 1621–1639.

Rietveld, A., Neutz, S., Simons, K., and Eaton, S. (1999). Association of sterol- and glycosylphosphatidylinositol-linked proteins with Drosophila raft lipid microdomains. J. Biol. Chem. *274*, 12049–12054.

Robinett, C.C., Giansanti, M.G., Gatti, M., and Fuller, M.T. (2009). TRAPPII is required for cleavage furrow ingression and localization of Rab11 in dividing male meiotic cells of Drosophila. J. Cell. Sci. *122*, 4526–4534.

Roelants, F.M., Breslow, D.K., Muir, A., Weissman, J.S., and Thorner, J. (2011). Protein kinase Ypk1 phosphorylates regulatory proteins Orm1 and Orm2 to control sphingolipid homeostasis in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA *108*, 19222–19227.

Sampaio, J.L., Gerl, M.J., Klose, C., Ejsing, C.S., Beug, H., Simons, K., and Shevchenko, A. (2011). Membrane lipidome of an epithelial cell line. Proc. Natl. Acad. Sci. USA *108*, 1903–1907.

Schonbaum, C.P., Lee, S., and Mahowald, A.P. (1995). The Drosophila yolkless gene encodes a vitellogenin receptor belonging to the low density lipoprotein receptor superfamily. Proc. Natl. Acad. Sci. USA *92*, 1485–1489.

Seppo, A., Moreland, M., Schweingruber, H., and Tiemeyer, M. (2000). Zwitterionic and acidic glycosphingolipids of the Drosophila melanogaster embryo. Eur. J. Biochem. *267*, 3549–3558.

Shui, G., Guan, X.L., Gopalakrishnan, P., Xue, Y., Goh, J.S., Yang, H., and Wenk, M.R. (2010a). Characterization of substrate preference for Slc1p and Cst26p in Saccharomyces cerevisiae using lipidomic approaches and an LPAAT activity assay. PLoS ONE *5*, e11956.

Shui, G., Guan, X.L., Low, C.P., Chua, G.H., Goh, J.S., Yang, H., and Wenk, M.R. (2010b). Toward one step analysis of cellular lipidomes using liquid chromatography coupled with mass spectrometry: application to Saccharomyces cerevisiae and Schizosaccharomyces pombe lipidomics. Mol. Biosyst. *6*, 1008–1017.

Simons, K., and Gerl, M.J. (2010). Revitalizing membrane rafts: new tools and insights. Nat. Rev. Mol. Cell Biol. *11*, 688–699.

Stark, W.S., Lin, T.N., Brackhahn, D., Christianson, J.S., and Sun, G.Y. (1993). Phospholipids in Drosophila heads: effects of visual mutants and phototransduction manipulations. Lipids *28*, 23–28.

Stiban, J., Tidhar, R., and Futerman, A.H. (2010). Ceramide synthases: roles in cell physiology and signaling. Adv. Exp. Med. Biol. *688*, 60–71.

Sullards, M.C., Liu, Y., Chen, Y., and Merrill, A.H., Jr. (2011). Analysis of mammalian sphingolipids by liquid chromatography tandem mass spectrometry (LC-MS/MS) and tissue imaging mass spectrometry (TIMS). Biochim. Biophys. Acta *1811*, 838–853.

Sullards, M.C., and Merrill, A.H., Jr. (2001). Analysis of sphingosine 1-phosphate, ceramides, and other bioactive sphingolipids by high-performance liquid chromatography-tandem mass spectrometry. Sci. STKE *2001*, pl1.

Szafer-Glusman, E., Giansanti, M.G., Nishihama, R., Bolival, B., Pringle, J., Gatti, M., and Fuller, M.T. (2008). A role for very-long-chain fatty acids in furrow ingression during cytokinesis in Drosophila spermatocytes. Curr. Biol. *18*, 1426–1431.

Terashima, J., and Bownes, M. (2005). A microarray analysis of genes involved in relating egg production to nutritional intake in Drosophila melanogaster. Cell Death Differ. *12*, 429–440.

Ternes, P., Franke, S., Zähringer, U., Sperling, P., and Heinz, E. (2002). Identification and characterization of a sphingolipid delta 4-desaturase family. J. Biol. Chem. *277*, 25512–25518.

van Meer, G., Voelker, D.R., and Feigenson, G.W. (2008). Membrane lipids: where they are and how they behave. Nat. Rev. Mol. Cell Biol. *9*, 112–124.

Walker, A.K., Jacobs, R.L., Watts, J.L., Rottiers, V., Jiang, K., Finnegan, D.M., Shioda, T., Hansen, M., Yang, F., Niebergall, L.J., et al. (2011). A conserved SREBP-1/phosphatidylcholine feedback circuit regulates lipogenesis in metazoans. Cell *147*, 840–852.

Wang, C., and Huang, X. (2012). Lipid metabolism and Drosophila sperm development. Sci China Life Sci. *55*, 35–40.

Yew, J.Y., Dreisewerd, K., Luftmann, H., Müthing, J., Pohlentz, G., and Kravitz, E.A. (2009). A new male sex pheromone and novel cuticular cues for chemical communication in Drosophila. Curr. Biol. *19*, 1245–1254.

Yuan, C., Rao, R.P., Jesmin, N., Bamba, T., Nagashima, K., Pascual, A., Preat, T., Fukusaki, E., Acharya, U., and Acharya, J.K. (2011). CDase is a pan-ceramidase in Drosophila. Mol. Biol. Cell *22*, 33–43.